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THE EFFECT OF ORAL AND TOPICAL TETRACYCLINE ON ACNE SEVERITY AND ON SURFACE LIPID COMPOSITION

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Groups of 20 males of high-school age with moderate acne were treated with oral tetracycline (500 mg/day), topical tetracycline (0.5% solution applied twice daily), or placebo for 8 weeks. The two panels treated with tetracycline showed a significant and equivalent reduction in acne severity as assessed by visual grading.

The surface lipids in the panel treated with oral tetracycline showed a small but not statistically significant decrease in free fatty acid content, but the subjects receiving topical tetracycline showed no reduction in free fatty acids. Further, neither treatment was associated with a change in mass of surface lipid nor did the mass or weight percent of any component of the surface lipids change with the decrease in acne severity. These results show that acne severity can be reduced with tetracycline (both oral and topical) without any concomitant quantitative change in surface lipids.

Skin surface lipids have been studied extensively in attempts to define their role in the etiology of acne. The component of skin surface lipid most frequently implicated as a causative agent in acne is the free fatty acid (FFA) fraction. Several workers have attempted to establish a correlation between skin surface lipid FFA content and acne severity with results that are quite variable. For example, Powell and Beveridge [1] and Runkel et al [2] were unable to detect any difference in FFA concentrations in skin lipids from acne patients and age peers without acne. Gloor et al [3] have reported lower FFA levels in lipids from the backs of teen-agers with severe acne than from subjects with moderate or mild acne. In contrast, Nicolaides et al [4] have shown that comedo lipids have a higher FFA content than do surface lipids at the same site, and Nicolaides et al [5] and Anderson et al [6] have shown that follicular lipids contain more FFA than surface lipids from the same site.

In addition to measuring the FFA content of surface lipids, investigators have attempted to demonstrate that medications which improve acne do reduce the FFA concentrations in surface lipids. Beveridge and Powell [7], for example, have reported that oral tetracycline causes a 50% decrease in surface lipid FFA levels in acne patients. Marples et al [8] obtained similar results with scalp lipids of some subjects given oral demethylchlortetracycline, and Kraus [9] showed that tetracycline applied topically in dimethylacetamide reduced the FFA content of surface lipids. Recently Fulton [10] reported that several antibiotics applied topically (2% in cream) can reduce surface lipid FFA/fatty ester ratio and can also reduce acne severity, and Gloor et al [11] have presented evidence that 5% tetracycline applied topically can reduce the FFA/triglyceride ratio in surface lipids of acne patients. In addition, it has been shown that β-naphthol can reduce surface FFA levels [12]. In contrast, Gloor et al [13] showed an insignificant reduction in FFA concentrations in the lipids from the backs of subjects treated with 100 mg tetracycline per day, but did see a significant reduction with 1000 mg tetracycline per day.

In the above-cited work, only that of Beveridge and Powell [7] and Gloor et al [11,13] examined the effects of tetracycline in subjects with acne and attempted to correlate changes in FFA levels with improvement in acne. The present work was undertaken in order to assess the effects of topically applied tetracycline and oral tetracycline on surface lipid composition and improvement in acne.

MATERIALS AND METHODS

Sixty males of high-school age, with acne of moderate severity, were divided randomly into three groups of 20. All subjects were examined weekly for 12 consecutive weeks. Three judges graded each subject according to the following procedure:

1. Pustules were counted between the hairline and the mandibular ridge from ear to ear (butterfly area).

2. A grade from 0 to 9 (none to severe) was assigned individually for comedones, papules, and macules based on an estimate of the fraction of the area affected by each of these lesions.

3. An overall grade from 0 to 8 (none to severe) was assigned. This grade was designed to correspond to the

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Abbreviations: C_{10}MSO = decylmethylsulfoxide, synthesized in house and greater than 99% pure; SMO = sucrose mono-oleate, synthesized in house and greater than 99% pure.

impression a subject would make on meeting a stranger at a distance of 2½ meters. The graders were trained with a set of standard photographs selected to make the severity scale linear.

The subjects were prescreened and no subject with cystic acne or a severity grade of less than 3 was included in this test. The grades assigned to each subject at each grading session were averaged and analyzed by the analysis of variance method. In this report we will present only the severity grades since they reflect the overall change in acne. It should be noted that both oral and topical tetracycline produced comparable changes in the individual lesion types. That is, these treatments produced a statistically significant reduction in pustules and papules but a nonsignificant decrease in comedones and macules.

Twice during the four weeks prior to the initiation of treatment, surface lipids of each subject were collected by the following method: a flat, glass extraction vessel, 17.5 cm^2 in area, resembling a Petri dish with a neck for solvent addition and a stopcock for draining, was placed against the right cheek. About 10 ml of diethyl ether was introduced into the vessel through the glass tube at the top. The ether remained in contact with the skin for 30 sec after which it was drained through the stopcock into a tared weighing bottle. The vessel was immediately refilled, and after 30 sec, drained into the same weighing bottle. This sample was blown to dryness under a stream of N_2. The residue was suspended in chloroform (~5 ml), centrifuged to separate solids, decanted, and again evaporated to dryness under N_2. The lipid residue was weighed.

At the end of the 4th week, the members of each panel began an 8-week treatment program as follows:

Panel I: The butterfly surface of the face was treated with a roll-on applicator daily with a 0.5% solution of tetracycline HCl in 30:70 ethanol:water (vol/vol) with 0.5% C_{10}MSO and 0.25% SMO. Each subject was given a 250-mg gelatin capsule of lactose twice daily.

Panel II: The butterfly surface of the face was treated twice daily with 30:70 ethanol:water solution. Each subject was given a 250-mg gelatin capsule of tetracycline HCl twice daily.

Panel III: The butterfly surface of the face was treated twice daily with 30:70 ethanol:water solution. Each subject was given a 250-mg gelatin capsule of lactose twice daily.

The lactose and tetracycline capsules were indistinguishable from one another. The topical placebo contained a vegetable dye (FD&C #5) to give a color similar to the tetracycline HCl solution. Neither the subjects or the graders were aware of the treatment assignments.

Following the procedure outlined above, surface lipids were collected four more times from each subject at 14-day intervals during the treatment period. No treatments were applied on the morning when lipid samples were to be obtained.

The second control period sample and the second and fourth treatment period samples from each subject were analyzed for weight percent FFA [14] and weight percent triglyceride [15] on an autoanalyzer. Only data from subjects present at both of the lipid sampling periods are included in this report.

The effects of the treatments on the lipid parameters (mass of lipid, weight percent of FFA, weight percent of TG, and mass of FFA) were evaluated by the analysis of variance method [16], and all possible correlations between lipid values and acne severity were determined [16].

Semiquantitative analysis of the composiiton of the surface lipids was accomplished by a modification of the thin-layer chromatography (TLC) technique described by Anderson et al [6]. In order to assess the content of five components in surface lipids, the TLC plates were successively developed in three solvent systems: (1) heptane to top of the plate, (2) benzene:hexane (80:20) to top of the plate, (3) hexane:ether:formic acid (70:30:2.5) to 7 cm from the origin. Development was followed by charring, and densitometer scanning as described [6]. The data are reported as uncorrected area percent for each lipid component.

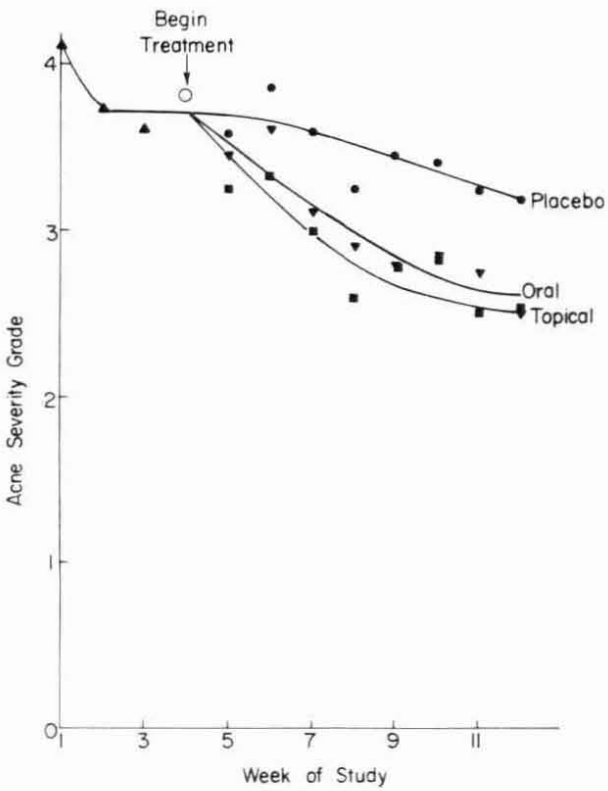

FIG. 1. Acne severity grades as a function of treatment time. First four points are the average of all subjects and remainder of points are the individual treatment group averages. The treatments used and grading technique are described in *Materials and Methods*.

TABLE I. *Effect of oral and topical tetracycline treatment on acne improvement*

Treatment	Change in acne severity[a]		
	>1 Unit decrease	<1 Decrease	No change or increase
Placebo (17)[b]	6	4	7
Oral tetracycline (18)	12	1	5
Topical tetracycline (19)	14	3	2

[a] Pretreatment grade minus final grade after 8 weeks of treatment

[b] Number in parentheses is number of subjects in each panel sampled for lipid analyses and acne grading at both the pretreatment time and after 8 weeks of treatment

RESULTS

To justify an assessment of the effects of acne therapies on skin surface lipid changes that may accompany alleviation of acne severity, it is essential to establish that the treatments employed do indeed decrease acne severity. Figure 1 depicts the changes in acne severity obtained with oral and topical tetracycline as a function of treatment time, compared with changes in a control group. All of the groups showed a decrease in severity with treatment, but the subjects treated with tetracycline (either oral or topical) showed more than twice the improvement attained in the group treated with both the oral placebo and the topical placebo (~1.2 severity units vs ~0.5 severity units). The decrease in severity grade in treatment groups was statistically significant from week 7 to the end of the study. There was no statistically significant difference in changes obtained with topical and oral tetracycline therapy.

The number of subjects on each panel who showed a decrease in acne severity (>1 unit), a slight decrease in severity (<1 unit), and no change or an increase in severity is presented in Table I. These data show that ~67% of the subjects treated with oral tetracycline (12/18) and ~74% of the subjects treated with topical tetracycline (14/19) showed a decrease in severity (>1 grade unit) compared to only ~35% of the subjects in the placebo group (6/17).

The surface lipid mass, and free fatty acid (FFA) concentrations before treatment and after 8 weeks of treatment are presented in Table II along with the corresponding changes in acne severity grades. The weight percent and mass of FFA in the surface lipids in the placebo panel did not change. The subjects treated with both oral tetracycline and topical tetracycline showed a decrease in FFA levels (both weight percent and mass). This decrease, however, was not statistically significant. Table II also shows that all groups had essentially the same mean weight percent and mass per unit area of FFA after 8 weeks of treatment and that the changes induced with tetracycline were a function of pretreatment mean differences and not post-treatment mean differences. Thus, in this study, treatment with tetracycline (either oral or topical) did not induce a statistically significant change in surface lipid weight percent or mass per unit area of FFA, but both treatments did produce a significant reduction in acne severity. Further, acne severity changes were not associated with changes in surface lipid mass (Tab II).

Since two recent publications [10,11] have reported that topical antibiotic causes a decrease in the surface lipid FFA/ester ratio [10] and FFA/triglyceride (TG) ratio [11], we have made a similar comparison (Tab. III). The FFA/TG ratio was lower in all three groups after 8 weeks of treatment than before treatment. The decrease ob-

TABLE II. *Surface lipid and severity grades in subjects treated with oral or topical tetracycline*

Parameter	Treatment		
	Placebo (17)[a]	Oral (18)	Topical (19)
Acne severity			
Pretreatment[b]	3.77 ± 0.25[c]	3.72 ± 0.28	3.68 ± 0.29
Post-treatment[d]	3.22 ± 0.35	2.78 ± 0.40	2.60 ± 0.28
Change[e]	−0.56 ± 0.31	−0.97 ± 0.15	−1.08 ± 0.29
Lipid mass (mg/17.2 cm²)			
Pretreatment	3.16 ± 0.21	3.26 ± 0.26	3.60 ± 0.30
Post-treatment	3.68 ± 0.45	3.22 ± 0.24	3.36 ± 0.24
Change	+0.43 ± 0.46	−0.04 ± 0.18	−0.24 ± 0.23
Weight % FFA			
Pretreatment	12.9 ± 2.2	16.5 ± 2.7	13.3 ± 1.3
Post-treatment	12.9 ± 1.8	12.0 ± 1.2	12.1 ± 1.3
Change	+0.03 ± 0.05	−4.5 ± 2.2	−1.2 ± 1.0
Mg FFA/17.2 cm²			
Pretreatment	0.42 ± 0.09	0.54 ± 0.10	0.46 ± 0.05
Post-treatment	0.45 ± 0.08	0.40 ± 0.06	0.40 ± 0.05
Change	+0.03 ± 0.05	−0.14 ± 0.07	−0.07 ± 0.05

[a] Number in parentheses is number of subjects
[b] Average of values obtained during weeks 3 and 4 of the pretreatment period
[c] Each value is the mean ± standard error of the mean for the number of subjects indicated
[d] Average of values obtained after 8 weeks of treatment
[e] Mean ± SEM for individual changes, pretreatment minus post-treatment values

TABLE III. *Surface lipid free fatty acid/triglyceride (FFA/TG) ratios*

	Treatment		
	Placebo (17)[a]	Oral (18)	Topical (19)
Pretreatment[b]	0.294 ± 0.068	0.408 ± 0.094	0.271 ± 0.033
Post-treatment[c]	0.281 ± 0.057	0.245 ± 0.038	0.241 ± 0.039
Change[d]	+0.013 ± 0.026	+0.163 ± 0.063	+0.030 ± 0.027
Correlation coefficient between acne change and FFA/TG ratio change	0.121	−0.060	0.073

[a] Number in parentheses is number of subjects
[b] Mean ± SEM before treatment began
[c] Mean ± SEM after 8 weeks of therapy
[d] Mean ± SEM for individual changes pretreatment minus post-treatment values

served in the group treated with oral tetracycline was the decrease that was statistically significant ($p < 0.05$). It should be noted that the post-treatment FFA/TG ratios were approximately the same in all three groups and that the significant decrease observed in the group treated with oral tetracycline was a function of its higher pretreatment ratio and not a lower post-treatment ratio. When individual subjects were analyzed for a correlation between a change in acne severity and a decrease in FFA/TG ratio, none of the correlations even approached statistical significance.

TABLE IV. *Changes in FFA in surface lipids in subjects selected for change in acne severity*

Parameter	Change in severity[a]		
	>1 Decrease	<1 Decrease	No change or increase
Number of subjects	32	8	14
Wt % FFA[b]	−2.0±1.0	−0.8±1.5	−2.4±2.0
mg FFA/17.2 cm²[c]	−0.07±0.04	−0.01±0.05	−0.08±0.07

[a] Change in acne severity in all subjects; change in acne between second grading and final grade

[b] Change in wt % FFA; final value minus control period value; mean ± SEM

[c] Final value minus control period value; mean ± SEM

The data in Table II are the average changes in the treatment groups and thus may mask correlations between changes in acne severity and changes in surface lipid FFA content. We, therefore, have determined the changes in FFA content (weight percent) and concentration (mg per unit area of skin) in subjects from all groups who showed a decrease in severity (>1 unit), a slight decrease (<1 unit), and no decrease or an increase in severity (Tab. IV). The results show that the change in FFA content and concentration were similar in all groups and that the variation among subjects within each group was almost as great as the mean change.

The data above show that no change in surface lipid mass, FFA content, or FFA/TG ratio was observed that could be associated with a change in acne severity in the subjects studied. In order to assess whether changes occurred in other surface lipid components, all of the samples of surface

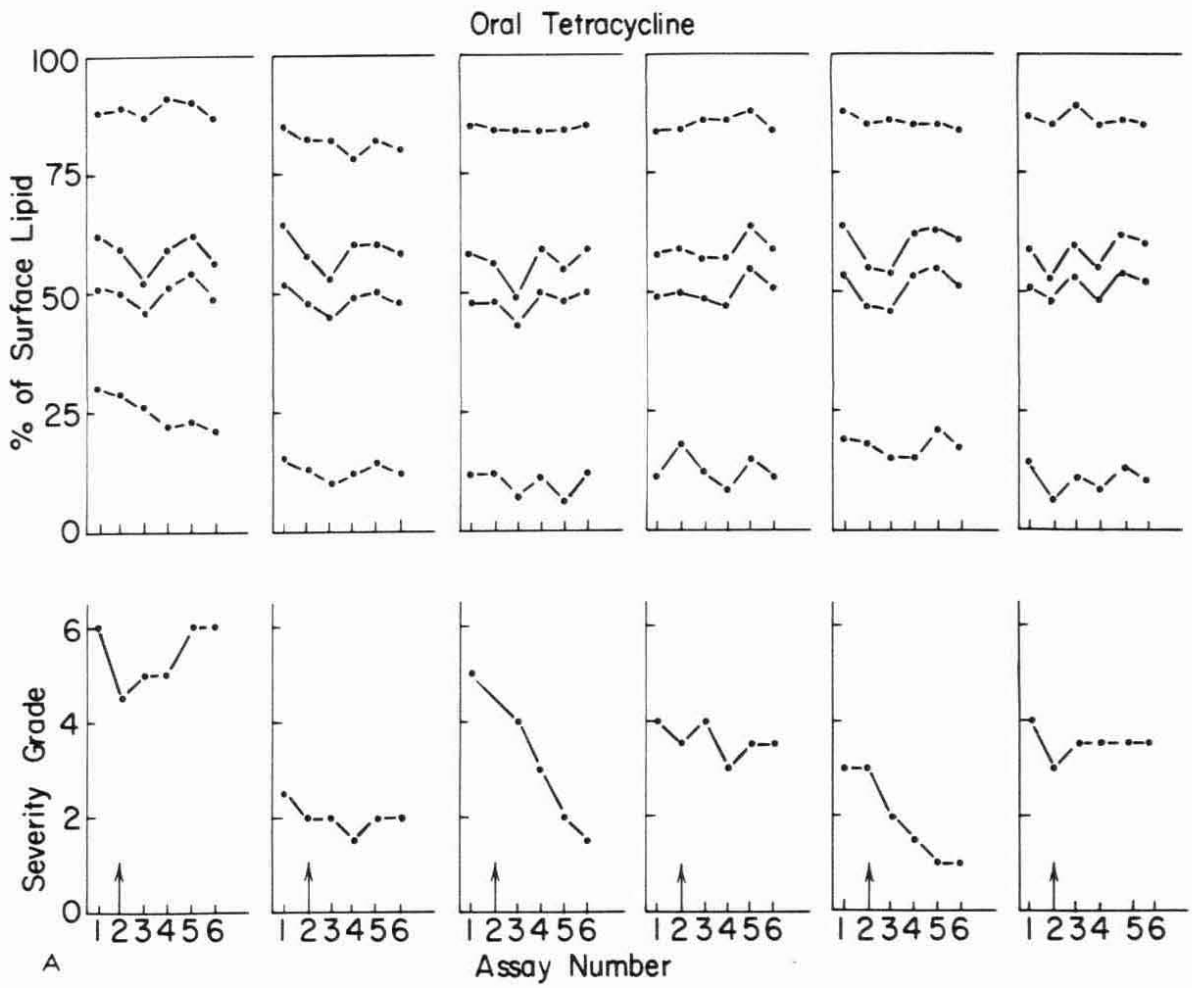

FIG. 2. Percentage composition of cheek surface lipids from subjects treated with oral tetracycline (*A*) and topical tetracycline (*B*). Abbreviations: Sq + HC = squalene + hydrocarbons; WE = wax esters; S + D = sterol + diglyceride; TG = triglyceride; FFA = free fatty acid.

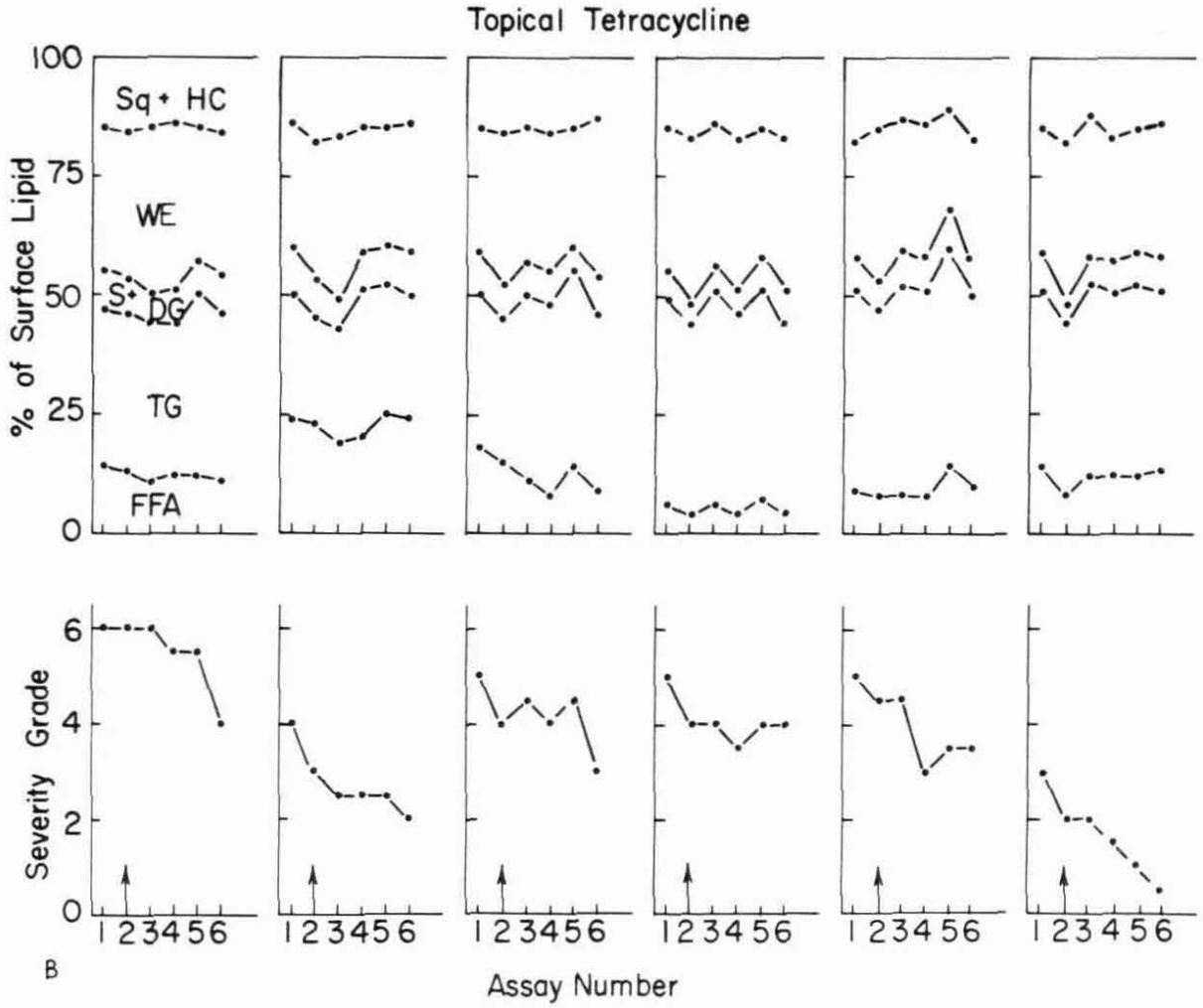

FIG. 2. B

lipids from 6 subjects treated with topical tetracycline and 6 subjects treated with oral tetracycline were examined by semiquantitative TLC (Fig. 2). In these 12 subjects there were no changes in any surface lipid component that could be correlated with changes in acne severity. That is, the composition of the surface lipids of each individual was constant whether the subject showed marked changes in acne severity or not. These results also show that initial acne severity grade does not seem to be related to the FFA content of the skin surface lipids.

DISCUSSION

The purpose of this investigation was to compare changes in acne severity to changes in cheek surface lipid composition. Data presented show that both oral and topical tetracycline caused a significant decrease in acne severity while the placebo treatment did not. Thus, we feel that we had a legitimate study in which to compare the changes in surface lipids with changes in acne severity.

The results of this work demonstrate that treatment for 8 weeks with 500 mg of tetracycline per day did not significantly reduce the FFA of the skin surface lipids. This appears to be at odds with data reported by Beveridge and Powell [7], Marples et al [8], and Gloor et al [13], all of whom report a decrease in surface lipid FFA concentrations with oral tetracycline. There are, however, several differences in the treatments among these studies. For example, Beveridge and Powell [7] sampled the forehead, Marples et al [8] the scalp, Gloor et al [13] the back, and we have examined the cheek. It is well documented that the FFA of surface lipids vary greatly at different sites on the body [17].

Another difference between our work and at least that of Beveridge and Powell [17] and Gloor et al [13] is that our surface lipid samples were casual samples, whereas these other authors used timed collections. Gloor et al [11] have reported, however, that topical treatment with 5% tetracycline reduces the FFA/TG ratio in surface lipids of both casual samples and timed collections of surface lipids.

The differences in results obtained in our work and that of others may also involve the severity of the acne of the subjects. In our work we have examined a cross-section of a high-school-age group; we had very few subjects with severe acne (>6 grade units) and the majority were in the 2 to

4 grade unit range. We cannot tell how these subjects compare to those used in other studies.

When subjects were treated with tetracycline topically, essentially no decrease in FFA concentration in the skin surface lipids was observed. This was an unexpected result since we had expected topical treatment to maximize the effect of tetracycline on skin flora and to reduce or inhibit bacterial lipase [18]. We have no ready explanation for the lack of effect of topical tetracycline on surface lipid composition, especially since other workers [9–11] have reported that antibiotics applied topically do reduce the FFA concentration in skin surface lipids. It is possible that the time between the last topical tetracycline treatment and lipid sampling (~18 hr) in our study was sufficient to allow lipolysis to recover. It is unlikely, however, that in all samples one would get recovery to the pretreatment value of FFA (Tab. II). It is also unlikely that if tetracycline were reducing the numbers of lipolytic bacteria [8] or inhibiting bacterial lipase [18], such treatment following 8 weeks of topical application would not have resulted in a measurable decrease in FFA concentration of the casual surface lipids.

Part of the objective of this work was to assess the effect of acne therapy on surface lipid composition and to test the hypothesis that acne is caused by FFA produced from sebum triglycerides by bacterial lipase [5]. While the data obtained do not negate this hypothesis, they do show that alleviation of acne by tetracycline need not be accompanied by a detectable decrease in surface lipid FFA levels of FFA/TG ratio. In fact, it was unexpected that topical tetracycline, which should have optimized the tetracycline effect against surface flora, had less effect on surface lipid FFA than oral tetracycline and yet the two treatments resulted in comparable reductions in acne severity. Thus, we would conclude that while FFA may be a causative agent in acne etiology, this effect cannot be ascertained by measuring casual surface lipid FFA levels.

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